Brown

The Composition of the Body Fat of the Common Woodchuck (Marmota Monax)

THE COMPOSITION OF THE BODY FAT OF THE COMMON WOODCHUCK, (MARMOTA MONAX)

BY

JOHN BERNIS BROWN

B. S. University of Illinois, 1915

THESIS

Submitted in Partial Fulfillment of the Requirements for the

Degree of

MASTER OF SCIENCE

IN CHEMISTRY

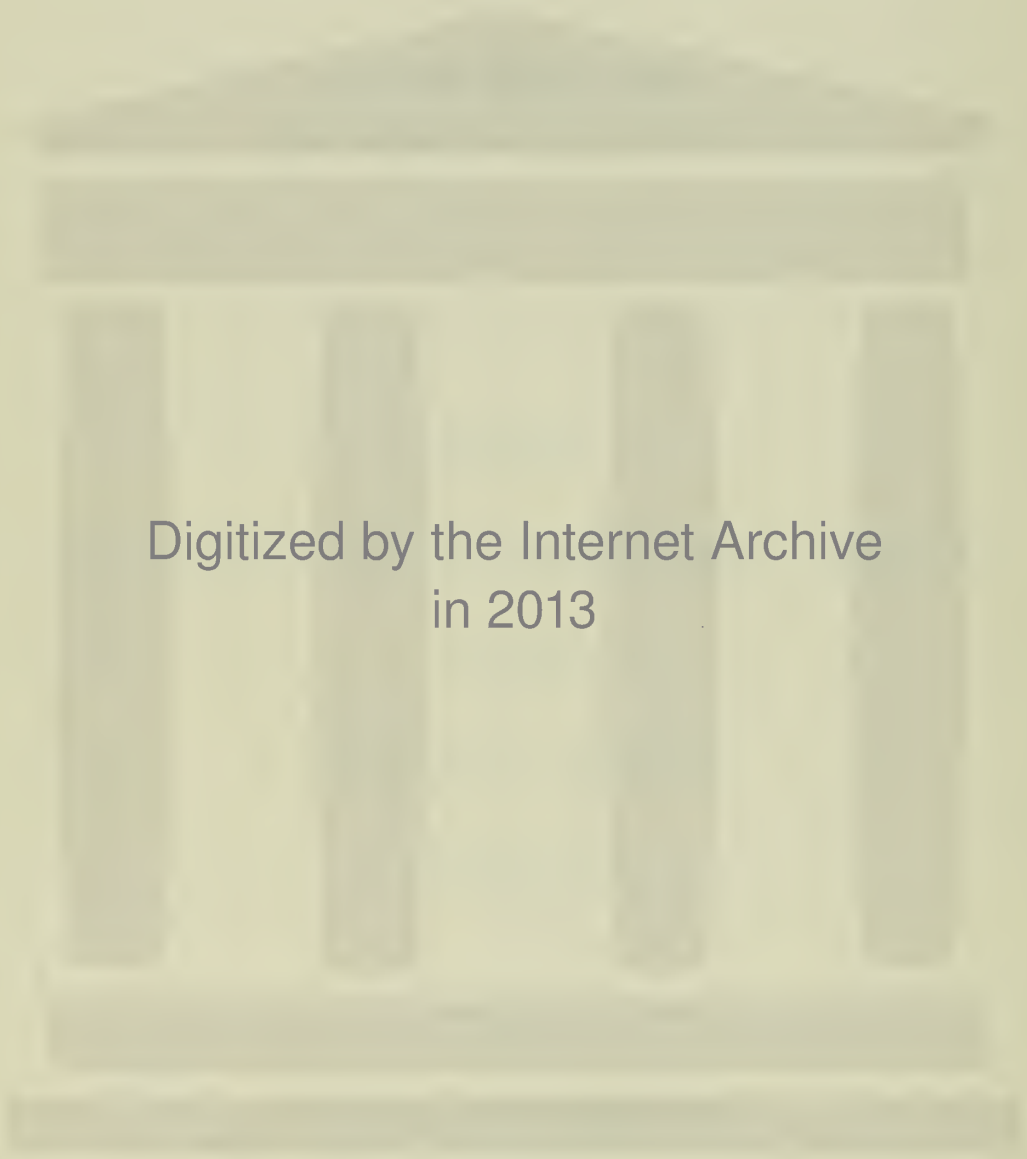
IN

THE GRADUATE SCHOOL

OF THE

UNIVERSITY OF ILLINOIS

1917



UNIVERSITY OF ILLINOIS

THE GRADUATE SCHOOL

May 28, 1917.

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY John Bernis Brown

ENTITLED The Composition of the Body Fat of the Common Woodchuck (Marmota Monax)

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science in Chemistry

Geo. D. Beal
In Charge of Thesis

W. A. Noyes
Head of Department

Recommendation concurred in:*

Committee on Final Examination*

*Required for doctor's degree but not for master's.

TABLE OF CONTENTS.

THE COMPOSITION OF THE BODY FAT OF THE COMMON WOODCHUCK (MARMOTA MONAX)

1. INTRODUCTION.

The common woodchuck (Marmota Monax) is a species of marmot occurring over wide areas in North America. The particular sub-species with which this paper is concerned is called the Marmota Monax Monax, and is found throughout most of Iowa, Arkansas, Illinois, Indiana, and the states east of these to the Atlantic ocean. In Illinois the woodchuck, or ground hog, is one of the most common of the larger animals which still run wild. In many localities there are such numbers of them that they afford a serious menace to crops and are a source of constant danger to farmers working in the field on account of the large holes which they dig. An average woodchuck weighs from four to five kilos. The flesh is edible, but is seldom used for that purpose. Although the skin has practically no value as a fur, it may however be made into good leather. In some counties bounties have been offered to reduce the number with little success. The fact that these animals possess such a large amount of body fat was brought to the attention of the author, and a thorough analysis was deemed advisable, especially since no record of any preceding analyses could be found.

Since the woodchuck furnishes one of the best examples of an hibernating animal, it was determined, also, to get a sample of the fat after hibernation to see if any change took

place during that peculiar period. Two samples were obtained for the present investigation. The first of these animals was killed about the middle of August. It was a large specimen weighing over five and a half kilos. The animal was living under ideal conditions in an alfalfa field, and when found, its stomach was gorged with alfalfa leaves. The second specimen, obtained the first week of the following April, was a female weighing about four kilos. Upon examination she was found to contain seven small embryos, each about five centimeters in length. The stomach was full of green moss, the only green vegetation available. Further details as to the fat will be given later. A general description of the marmots, their habits, food, etc., may be obtained in "North American Fauna, No. 37."(1)

The only analytical data on the fat of the general class of Marmots is mentioned by Lewkowitsch.(2) The species here described is that of the Arctomys Marmota, which occurs in Europe, and is said not to resemble the American variety to any great extent. A table comparing the analysis of this with that of the above samples will be given later.

II. PREPARATION OF SAMPLES.

On account of the size of the animals, complete extraction of the fat was not feasable. The greater portion of the body fat was obtained mechanically, however, by skinning the animal, scraping off all of the layers of fat lying under the skin, and removing by means of a knife the large masses of fat from the intestinal region. Both specimens were treated as

nearly as possible in the same way by one person, so that there is no reason for the samples differing because of the method of extraction. The fatty material thus obtained was boiled for about twenty minutes with a little water, and expressed by hand power while still hot. The oil was then freed from water as much as possible, and filtered to remove the last traces of moisture and foreign matter. The product thus obtained, which may be called an oil because it is a liquid at ordinary temperature, varied slightly in appearance in the two samples. For convenience the oil obtained in late summer from the large animal will be designated as (A) and the one taken after hibernation as (B). Sample (A) was almost colorless with a slightly yellow tint, and weighed over six hundred grams. Sample (B) was considerably more yellowish in color, and weighed only about one hundred and seventy grams. Both deposited stearin on standing for some time at 22° C. This disappeared on warming. The following table shows the physical constants of the oils.

Sample.	Index of Refraction	Butyro-Ref. Calculated	Temp. of turbidity	Temp. of solution	Sp. g. 23°
(A)	1.4726 (20°)	70.5	5.5°	12-18°	0.9122
(B)	1.4704 "	67.0	6.5°	21-23°	0.9099

III. GENERAL CHEMICAL ANALYSIS.

The general methods for the analysis were taken from Bulletin 107 of the Bureau of Chemistry.(3) Determinations were made of the saponification number, soluble and insoluble fatty acids, unsaponifiable material, Hehner number, solid and

liquid fatty acids, their iodine number, etc. The description and general discussion of these methods, with improvements where possible, will follow.

Saponification Number.- The saponification number is the number of milligrams of potassium hydroxide required to saponify one gram of the fat. It is determined by weighing out about 5 g of the fat into a 250 c.c. Erlenmeyer flask, adding 50 c.c. of alcoholic potash, refluxing about thirty minutes, or until the saponification is complete, and then titrating the excess of alkali with standard hydrochloric acid. (a) A blank determination is made at the same time on the alcoholic potash. The number of c.c. required to neutralise the excess KOH is subtracted from the amount required by the blank. This gives the amount of KOH, in terms of HCl, required to saponify the fat. From this data and the normality of the acid the number of milligrams of KOH required to saponify one gram of fat may be calculated. The results were as follows:

Sample.		c.c. HCl for blank	c.c. HCl for excess	c.c. HCl used by sample	Saponification number
(A).	(1) 4.5256	55.04	25.67	29.37	196.69
	(2) 4.4501	55.04	26.20	28.84	196.45
				Ave.=	196.57
(B).	(1) 8.5030	64.97	10.32	54.65	194.81
	(2) 5.7543	49.30	12.53	36.77	193.68
	(3) 6.2232	49.30	9.50	39.80	193.86
				Ave.=	194.12

(a)
Note.- The normality factors for the standard acid and alkali used throughout this investigation are given here and will not be repeated each time mentioned.

Hydrochloric acid = 0.5401 Normal.
Sodium hydroxide = 0.2143 " (Carbonate free)

Insoluble Fatty Acids.- Since the method given in Bulletin 107 was not followed, and since the method described in this paper was considered more satisfactory, especially with this fat, the former is reprinted as follows: " Allow the flask containing the cake of insoluble fatty acids from the preceding determination, and the paper through which the soluble fatty acids have been filtered, to drain and dry for twelve hours. Transfer the cake, together with as much of the fatty acids as can be removed from the filter paper, to a weighed glass evaporating dish. Then place the funnel, with the filter, in the Erlenmeyer flask, and thoroughly wash the paper with absolute alcohol. Rinse the flask with the washings from the filter paper, then with pure alcohol, and transfer the filtrate and washings to the evaporating dish. Keep the dish on the steam bath until the alcohol is evaporated, dry for two hours at 100° C., cool in a dessicator, and weigh. Again place in the air bath as before, cool, and weigh. If there is any considerable decrease in weight, repeat. " The following objections to this method are evident:

(1). Too much time is involved in draining and drying the cake.

(2). The use of absolute alcohol is often an obstacle.

(3). Extended heating at 100°, in air especially, causes great oxidation of the acids, and subsequent error.

The following method was used with considerable success, and practically eliminated the above objections. The cake was prepared as usual. After two washings with water, the acids on the filter were washed into the flask with ether. A gram or two of anhydrous sodium sulphate was then added to the contents

of the flask, which absorbs the few drops of water which have clung to the sides of the flask. The ether solution is then filtered into a weighed flask , and the sodium sulphate, flask, and filter washed with small portions of ether to remove the last traces of acids. Most of the ether may be evaporated off with a condenser to collect and save the ether. The last two or three cubic centimeters are evaporated, however, in a boiling water oven through which a current of carbon dioxide is constantly passing. After drying thus for half an hour, the last traces of ether are usually gone, and the flask is weighed, after cooling and washing out the heavy CO_2 with air. From the weight of the acids the percentage of insoluble acids is readily calculated, and samples may be at once weighed out for determining the iodine number and mean molecular weight.

Sample		Wt. Fatty Acids	% Insol. Acids
(A).	(1) 3.4933	3.3080	94.69
	(2) 3.4142	3.2152	94.17
			Ave.= 94.43
(B).	(1) 6.2232	5.9305	95.30
	(2) 4.2971	4.0059	93.44 (Low)
	(3) 5.7543	5.4969	95.53
			Ave.(1)-(3) 95.41

Iodine Number of Above.- This was determined as described later under the determination of the iodine number of the oil. Samples were weighed out directly from the five samples given above. It was found by experience that standing for any length of time before the determination was made caused a very appreciable lowering of the iodine number. Consequently the following work was done as nearly as possible on the same day

that the fatty acids were liberated.

Sample		Blank $Na_2S_2O_3$ (a)	Excess	c.c. used	Iodine No.
(A).(1)a.	0.2830	75.50	46.70	28.80	97.70
b.	0.3751	75.50	36.78	38.72	98.40
				Ave.=	98.05
(B).(1)a.	0.2092	52.87	35.36	17.51	80.35
b.	0.3524	52.87	22.70	30.17	82.19
(2)a.	0.2714	52.88	29.85	23.03	81.46
b.	0.3842	52.88	22.31	30.57	76.40 (Low)
(3)a.	0.2119	52.88	35.20	17.68	80.10
b.	0.2869	52.88	29.12	23.17	79.51
				Ave. omitting (2)b=	80.72

Mean Molecular Weight of Insoluble Acids.- This determination was made by titrating a sample of the acids with standard NaOH in boiling neutral alcohol. A blank was subtracted for the alcohol alone.

Sample		c.c. NaOH	Mean Mol. Wt.
(A).(1)a.	0.2000	3.61	260.00
b.	0.2000	3.60	260.70
		Ave. =	260.35 (Low)
(B).(1)a.	0.7798	13.12	277.35
b.	0.7600	12.70	279.24
(2)a.	0.5671	9.55	277.10
b.	0.7748	13.08	276.41
(3)a.	0.5027	8.38	279.94
b.	0.6523	10.86	280.30
		Ave. =	278.39

(a). The same standard sodium thiosulphate was used throughout this investigation. The normality factor is given in terms of iodine. 1 c.c. $Na_2S_2O_3$ = 0.0096 g iodine.

Soluble Fatty Acids.- The soluble acids were determined on the samples from the saponification number. The mixture of alcohol and water was evaporated off as far as possible, and in each case enough standard acid was added to afford an excess of one cubic centimeter of the HCl, after all of the fatty acids had been liberated from the soaps. The mixture was then heated until the fatty acids collected in a clear layer on top. Hot water was added until the acids rose almost to the top of the neck of the flask, and the contents were then cooled in an ice bath. The excessive cooling was necessary because the fatty acids were almost liquid at room temperature. They would partly solidify at 25° C. on long standing. Boiled water was used in the dilution in order not to have CO_2 present in the subsequent titration. The water solution of the soluble acids thus obtained was poured carefully through a filter, taking care to keep the acid cake in the flask. The process of washing with hot water was then repeated twice. The combined filtrates were then titrated with standard NaOH with phenolphthalein as an indicator. The total alkali used was corrected for the excess HCl which had been added, and the soluble acids were calculated as butyric acid.

Sample		c.c. NaOH used	% Butyric acid
(A).(1)	6.4248	2.46	0.72
(2)	4.6188	1.59	0.65
			Ave.= 0.68
(B).(1)	5.7543	0.10	Practically none.

From the odor of the filtrates there was no evidence of the presence of any butyric acid. There was a distinct odor, however, of the intermediate higher acids in (A), such as

caprylic acid, for example. Not enough material was available to actually isolate and prove the presence of the individual soluble acids. In case caprylic acid were present, it is evident that the percentage would be about double that calculated for butyric acid, or well over 1 % .

Acid Number.- About ten grams of fat was dissolved in 50 c.c. of neutral alcohol, and titrated boiling hot with NaOH. A blank was run at the same time on another sample of the alcohol. The difference between the number of cubic centimeters of NaOH required by the fat and that of the blank gave the number of cubic centimeters required by the free fatty acids in the fat. The acid number is the number of milligrams of KOH required to neutralise the free fatty acids in one gram of fat.

Sample		c.c. NaOH used	Acid No.	% oleic acid
(A).(1)	9.7407	0.485	0.60	0.30
(2)	10.0643	0.51	0.61	0.30

(B). Not determined.

Unsaponifiable Material.- The unsaponifiable material usually consists of alcohols of high molecular weight, and was determined on the above samples by extraction with ether of the saponified fat from which the alcohol had been removed by evaporation. The ether from this extraction was evaporated in a drying oven in a weighed beaker, and the increase in weight of the beaker taken as the unsaponifiable.

Sample		Wt. unsaponifiable	% unsaponifiable
(A).(1)	9.7407	0.0237	0.24
(2)	10.0643	0.0223	0.22

Reichert-Meissl Number.- About 5 g of fat was saponified with glycerol-soda by heating the mixture over a naked flame until clear. While still hot 135 c.c. of boiled distilled water was added, slowly at first to prevent foaming, and then 5 c.c. of dilute sulphuric acid (made by diluting 200 c.c. of concentrated acid with a liter of water). Pumice stone was added to prevent bumping, and the whole was heated to boiling, and the distillate collected in a 100 c.c. volumetric flask. Then 10 c.c. more was collected, and the whole distillate was mixed thoroughly by shaking. The distillation was so regulated as to collect 110 c.c. in about thirty minutes. The distillate was poured through a dry filter, and 100 c.c. of the filtrate titrated with standard NaOH with phenolphthalein as an indicator. The number of cubic centimeters of alkali was increased by one-tenth. The Reichert-Meissl number is the number of cubic centimeters of tenth normal alkali required to neutralise the volatile fatty acids from five grams of fat.

Sample		c.c. NaOH used	c.c. NaOH used for 5 g	Reichert-Meissl number
(A).(1)	3.4560	0.32	0.46	0.98
(2)	4.5920	0.60	0.63	1.34
(3)	4.4521	0.61	0.68	1.44
			Ave. =	1.28

Iodine Number of Oil.- The Hanus method was used in all of the determinations throughout this investigation. A brief description will be given here and will suffice for the other determinations. About 0.2 g of the fat or fatty acid was weighed into a glass stoppered flask, and 10 c.c. of

chloroform added. When the oil had gone into solution, the iodine solution of iodine monobromide in glacial acetic acid was added from a standardized pipette, usually 25 c.c. At the same time a blank was run in another flask without any oil. Both flasks were sealed by pouring a little glacial acetic acid over the glass stopper. After standing exactly thirty minutes, 10 c.c. of potassium iodide solution was added in such a manner that the glass stopper was thoroughly washed. After shaking and diluting standard thiosulphate was added from a burette, and the excess of iodine titrated in this manner with starch as an indicator. The number of cubic centimeters of thiosulphate required for this was subtracted from the volume of thiosulphate required by the blank, and from this data and the normality factor of the sodium thiosulphate the iodine number, or percentage of iodine, absorbed by the oil was calculated. It is obvious that this determination is very important, because it is a direct measure of the degree of unsaturation of the oil.

Sample		Blank $Na_2S_2O_3$	Excess	c.c. used by oil	Iodine No.
(A).(1)	0.4236	69.52	24.55	44.97	101.91
(2)	0.1574	69.12	52.42	16.70	101.87
(3)	0.1547	69.12	52.67	16.45	102.18
				Ave.=	101.98
(B).(1)	0.2527	53.30	31.60	21.70	82.44
(2)	0.3298	53.30	25.25	28.11	81.82
(3)	0.3561	61.60	31.16	30.44	82.06
				Ave. =	82.11

Liquid Fatty Acids.- Fatty acids may be divided into two classes, the saturated and unsaturated. The more important saturated acids are palmitic and stearic, while the unsaturated are best characterised by oleic, linolic, and linolenic acids, having one, two, and three double bonds respectively. There are many methods of separating these two groups from each other roughly, but practically no method is by any means accurate from the quantitative point of view. By far the best method known at present is that of Gusserow and Varrentrapp, or better known as the lead salt- ether method. (4) and (5)
The method followed for sample (A) was the customary Muter modification, but this was unsatisfactory for reasons which will be mentioned. In the procedure for sample (B) several changes were made as seemed advisable. The Muter method as carried out was mainly as follows: A sample of about 5 g of fat was weighed out in an Erlenmeyer flask, and saponified with alcoholic potash. The alcohol was then evaporated off on the steam bath, water was added, and heat was applied until the soap had dissolved to a clear solution. The excess alkali was then removed with dilute acetic acid, and while still hot, lead acetate was poured in. The soaps thus precipitated usually settled to a mass in the bottom, and on cooling and standing for a short time the supernatent liquid was poured off as completely as possible. The flask was inverted and allowed to drain completely, and then about 10 c.c. of alcohol was added, the flask shaken gently so as not to loosen the soap, and the alcohol poured off carefully. With care this was usually done without losing any appreciable

quantity of soap. About 150 c.c. of ethyl ether was then added, and the whole refluxed for an hour, or until practically all of the soaps had gone into solution, when the flask was removed and cooled to about 15° C. A fairly large precipitate of the lead soaps of the solid acids which are insoluble in ether came down here. Usually on standing and cooling the ether layer became clear, but in one or two cases a colloidal solution persisted. In case the latter happens, about the best thing to do is to start over again as it is practically impossible to get a good crystallization. The precipitate was filtered off, washed thoroly with cold ether, and used in determining the solid acids. The filtrate containing the lead salts of the liquid fatty acids was placed in a separatory funnel, hydrochloric acid added, and the two shaken thoroughly, causing a white precipitate of lead chloride to come down. This precipitate and the HCl layer were drawn off, and a fresh portion of the latter added, and the process repeated. The ether solution of the fatty acids liberated was then washed with a small volume of water to remove any HCl, and transferred to a Muter tube, which is merely a device to draw off aliquot portions for the various determinations. A sample of this solution was taken, dried in a hot water oven under CO_2 to prevent oxidation, and weighed. The percentage of total liquid fatty acids and their iodine value were determined.

Sample	Total vol. ether sol.	Aliquot portion	Wt. acids found	% liquid acids
(A).(1)a.	4.3620	208.00	62.3	0.9988	76.44
b.	"	"	53.8	0.8695	77.60
				Ave.=	77.02

Sample	Total vol. ether sol.	Aliquot portion	Wt. acids found	% liquid acids
(A).(2)a. 5.5172	252.5	72.0	1.2453	77.02
b. "	"	68.0	1.1490	77.33
			Ave.	=77.17
			Total Ave.	=77.10

Sample		c.c. thiosulphate used	Iodine No. of liquid fatty acids
(A).(1)	0.1703	20.46	115.35
(2)	0.2162	26.09	115.85
			Ave. = 115.60
(3)	0.1812	15.50	82.12
(4)	0.1925	16.58	82.68

Samples (3) and (4) are given to show the effect of standing for some time on the iodine number. The iodine numbers were taken on them two weeks after they had been prepared free from ether.

The determination of the liquid fatty acids in sample (B) was modified in some respects. The faults of the above method may be pointed out as follows: First, the ether extraction of the soaps should be done in the absence of water. The method as given does not remove all of the water. Secondly, the taking of aliquot portions always introduces errors, especially as in this case, where comparatively small volumes of ether must be measured in a Muter tube of large diameter. Thirdly, the ether solution in the tube is moist, and frequently introduces water into the fatty acids. The removal of this water in a water oven even by extended heating is almost impossible. And lastly, in shaking up the ether with water, enough of the ether goes into solution in the water, and is

consequently lost, to vitiate the results.

The first difficulty mentioned above was eliminated by adding several grams of anhydrous sodium sulphate to the ether extraction. This foreign substance does not affect the analysis in any respect. The second, third, and last objections were removed by not using the Muter tube at all, but proceeding as follows.- The lead soaps are decomposed as usual with HCl, using as small volumes of the acid as possible. The acid layer is drawn off, the ether dried with sodium sulphate, filtered into a tared flask, the filter paper and sulphate washed with small portions of fresh ether, and most of the ether is finally evaporated off on the water bath. When only a few cubic centimeters remain, the flask is placed in a water oven, and heated under CO_2. Generally about an hour in the oven is required to remove the last traces of ether. The flask is then reweighed, and by difference the amount of liquid acids is found. This procedure gave very satisfactory results in comparison with the Muter method.

Sample		Wt. acids	% liquid acids
(B).(1)	6.3943	4.9648	77.65
(2)	7.3962	5.5303	74.77

Sample	(From above)	Blank $Na_2S_2O_3$	Excess	c.c. used	Iodine No. liquid acids
(1)a.	0.2023	52.80	32.30	20.50	97.28
b.	0.2090	52.80	31.42	21.38	95.97
				Ave.=	96.62
(2)a.	0.3056	52.80	23.00	29.80	93.61
b.	0.2234	52.80	30.67	22.13	95.10

Sample (From above)		c.c. NaOH	Mean molecular weight of liquid acids
(1)a.	0.7272	12.12	279.98
b.	0.6116	10.17	280.63
		Ave. =	280.32
(2)a.	0.4926	8.25	278.63
b.	0.5478	9.16	279.07
		Ave. =	278.85

Saturated Fatty Acids.- The lead salts, obtained by filtering off the ether in the preceding determination, and any sodium sulphate which had been added for drying purposes, remaining on the filter, were washed back into the flask containing the remainder of the lead soaps of the saturated acids by piercing a hole in the filter paper and washing with boiling dilute HCl from a wash bottle. In this way all of the soaps could be removed nicely to the flask. The contents of the flask were then heated until the fatty acids collected in a clear layer on top, after which the flask was cooled, and the water poured off carefully through a filter as completely as possible. The acids which had unavoidably been poured onto the filter were washed back into the flask with ether, 100 c.c. of ether added, and the ether solution dried with sodium sulphate for a short time, after which the ether was filtered into a tared flask, and evaporated off with the usual precautions against oxidation. After weighing, samples were taken for the iodine number and mean molecular weight.

Sample		Wt. saturated acids	% saturated acids
(A).(1)	4.3620	0.7024	16.12
(2)	5.5172	0.9253	16.77
		Ave. =	16.45

Sample (From above)		c.c. thiosulphate used	Iodine No.
(1)	0.2997	3.83	12.26
(2)	0.3196	3.98	11.95
		Ave. =	12.09

Sample (From above)		c.c. NaOH used	Mean molecular weight
(1)	0.4027	7.20	262.4
(2)	0.6075	11.00	258.4
		Ave. =	260.4

Sample		Wt. saturated acids	% saturated acids
(B).(1)	6.3943	1.0513	16.41
(2)	7.3962	1.2751	17.24

Sample (From above)		Blank $Na_2S_2O_3$	Excess	c.c used	Iodine No.
(1)	0.2864	21.15	18.79	2.36	7.91
(2)	0.2900	21.15	17.77	3.38	11.19

Sample (From above)		c.c. NaOH used	Mean molecular weight
(1)	0.5158	9.13	263.63
(2)	0.6160	10.90	263.72

Sample (B) (2) was slightly overheated in the saponification so that it is probably a little in error. Consequently later calculations will be based on the determinations of (B) (1) alone.

Determination of Linolenic Acid.- Linolenic acid forms an ether-insoluble hexabromide on treating in the cold with an excess of bromine. Consequently it may be determined, at least somewhat better than qualitatively, by brominating the free fatty acids in ethyl ether for several hours at 0° C. An ether solution of the fatty acids, prepared as usual, was cooled to 0° C., and bromine was added slowly until its color

persisted after shaking for a few minutes. The mixture was allowed to stand for four hours or more at this temperature, the ether was filtered off, and the hexabromide was washed with fresh portions of cold ether. After drying in the water oven, the linolenic hexabromide was weighed. Several determinations gave greatly variant results. Fair checks were obtained, however, when samples were run side by side under exactly the same conditions. Although the method is far from satisfactory at best, it is nevertheless the only one of any value in determining linolenic acid.

Sample	Wt. hexabromide	% hexabromide	% linolenic acid
(A).(1) 4.8501	0.4230	8.72	3.20
(2) 7.5592	0.7878	10.42	3.83
		Ave. =	3.51
(B).(1) 11.5501	0.0572	Practically none.	

Elaidin Test.- Samples of the oils gave a butter-like elaidin on standing over night.

Valenta Test.- Both oils went into solution in glacial acetic acid between 60-70° C.

IV. CALCULATION OF THE INDIVIDUAL FATTY ACIDS.

From the data of the preceding determinations it is possible to calculate with fair approximation the percentages of the individual fatty acids. A thorough discussion of the methods for doing this is given in Lewkowitsch.(6) Accordingly the calculations will be given only in brief here. The actual percentages will be given as the percent of the total oil, not the fraction of the total fatty acid content. Under ideal

conditions, then, they should add up to the percentage of total fatty acids, as actually determined.

From the iodine value of the saturated acids, assuming all of the unsaturation to be due to oleic acid, the amount of oleic acid may be found from the following equation.-

$$\frac{\text{Iodine No}}{90.07} \times \text{\% sat. acids} = \text{\% oleic acid}$$

or

Sample (A) $\frac{12.09}{90.07} \times 16.45 = 2.21$ % oleic acid.

Sample (B) $\frac{7.91}{90.07} \times 16.41 = 1.44$ % oleic acid.

On observing the mean molecular weight of the solid acids in (A) it is found that it is practically correct for a mixture of 2.21 % oleic acid, and 14.44 % of palmitic acid. Consequently stearic acid is probably absent. Under the same conditions in (B), however, a trial calculation shows the presence of about equal quantities of stearic and oleic acids. As they differ only two points in the molecular weight, it is possible to calculate the percentages from the following simultaneous equations.-

x plus $y = 100$

$M' x$ plus $M'' y = 100\ M$

where x equals the combined percentage of oleic and stearic acids, of molecular weight, $M' = 283$, and y is the percentage of palmitic acid, molecular weight, $M'' = 256$. M is the mean molecular weight of the sample.

Substituting in the simultaneous equations, solving for x and y, and reducing these values to 16.41 instead of 100, the combined percentage of oleic and stearic acids is found to be

2.26, and of y by difference 14.15. Consequently in sample (B) there is 14.15 % of palmitic acid, 1.44 % of oleic acid, and 0.82 % of stearic acid.

The calculation of the individual acids in the liquid acids is more difficult, as there is no means of determining the amount of solid acids which inevitably go into solution in the lead salt-ether separation. The amount of linolenic acid has been determined by a different process. There remains to find the amount of oleic and linolic acids present. The total amounts of liquid acids were in (A) 77.10 % of iodine number 115.60, and in (B) 77.65 % of iodine number 96.62. Sample (A) contains 3.51 % of linolenic acid, corresponding to an iodine value of 12.34, leaving 103.26 for the iodine absorption of the oleic and linolic acids, and 73.59 for the combined percentage of the two. If then x equals the part of the whole of oleic, and y equals the part of the whole of linolic, I being their combined iodine value, the following equations follow.-

x plus y = 100

90.07 x plus 181.42 y = 100 I

Substituting and solving for the two, and reducing to 73.59 parts, it is found that there is 10.62 % of linolic and 62.97 % of oleic acid present. Adding the last result to the amount of oleic acid found in the saturated acids, the total percentage of oleic acid is found to be 65.18.

In Sample (B), since it is probable that no more than a trace of linolenic acid is present, the last step may be resorted to immediately. Working from the same equations as

given above, the percentage of oleic acid in the liquid acids is determined as 72.08, and of linolic acid as 5.57. The total percentage of oleic acid is 73.52. In order to sum up the percentages of the different acids in both samples, the following table is given.-

Acid	Sample (A)	(B)
Oleic	65.18	73.52
Linolic	10.62	5.57
Linolenic	3.51	Trace?
Palmitic	14.44	14.15
Stearic	00.00	0.82
Soluble(Butyric)	0.70	0.00
Total	94.45	94.06

V. TABLE OF ANALYTICAL DATA ON WOODCHUCK OIL.

	(A)	(B)	Arctomys Marmota (7)
Specific Gravity(23°)	0.9122	0.9099	0.9183
Solidifying Point	5.5°	6.5°	---
Melting Point	12-18°	21-23°	---
Saponification No.	196.57	194.12	197.1
Reichert-Meissl No.	1.28	---	0.60
Iodine Value	101.98	82.11	109.1
Soluble Acids	0.70	none	---
Insoluble Acids	94.43	95.41	95.8
" " Iodine No.	98.05	80.72	105.6
Unsaponifiable	0.23	---	---
Refractive Index(20°)	1.4726	1.4704	---
Elaidin Test	Paste	Paste	---

VI. CONCLUSIONS.

Since oils from different specimens of the same animal vary to a certain degree, the analysis of samples of two different oils can not be used to prove absolutely any one fact. The following remarks, however, may be made with a fair degree of certainty.-

1. The body fat of the woodchuck belongs to the general class of semi-drying animal oils.
2. The oil taken before hibernation differed considerably from that taken after. The former was less solid, had a higher iodine value, and contained notable quantities of the soluble acids and linolenic acid, while the latter contained no linolenic or soluble acids, and also less linolic acid. The free acids of the latter solidified quite completely at room temperature, while those of the former remained partly liquid under those conditions.
3. The animal killed before hibernation had from three to four times as much body fat as the one taken in the spring.
4. No positive statements may be made as to whether the change in composition of the fat was due to hiberna tion or to some other factor. It is possible that when the body begins its consumption of reserve fat during a condition of hibernation the glycerides of the soluble and of the more highly unsaturated acids are taken first, or at least in larger proportion at first.
5. The chemical composition of ordinary woodchuck fat under normal conditions is quite similar to that of the Arctomys Marmota of Europe.

REFERENCES.

(1). North American Fauna, No. 37, " Revision of the American Marmots.

(2). Lewkowitsch, " Technology of Oils, Fats, and Waxes" Vol. II, 5th Ed., p. 674.

(3). Bulletin 107 (Revised), Bureau of Chemistry.

(4). Liebig's Annal., 1828 (27), 153.

(5). ibid 1840 (35), 197.

(6). Lewkowitsch, " Technology of Oils, Fats, and Waxes." Vol. I, 5th Ed., Chapters VIII and XI.

(7). Same as (2).